

1 **Supplementary Information**

2

3 **A novel and efficient platform for discovering non-canonical quorum-quenching**
4 **proteins**

5 Jinxing Liao^a, Zihan Li^a, Dan Xiong^a, Danyu Shen^a, Lu Wang^b, Xiaolong Shao^a, Tao
6 Li^{c*}, Guoliang Qian^{a*}

7

8

9 This file contains 4 supplemental tables and 17 supplemental figures.

1 **Table S1 Gene candidates identified in *L. enzymogenes* for quenching AHL quorum sensing**

Gene ID	Functional annotation
Le0007	heparinase III protein [Pedobacter heparinus]
Le0012	hypothetical protein [Rudaea cellulositytica]
Le0022	NA
Le0040	NA
Le0060	hypothetical protein [Dyella japonica]
Le0081	Muramidase-2 [Stenotrophomonas maltophilia]
Le0095	hypothetical protein [Variovorax paradoxus EPS, Varpa_3397]
Le0096	low temperature requirement A protein LtrA [Cellvibrio sp. BR]
Le0100	NA
Le0101	hypothetical protein [Dyella japonica]
Le0138	hypothetical protein [Xanthomonas axonopodis]
Le0139	hypothetical protein [Chondromyces apiculatus Myxococcus sp.]
Le0141	hypothetical protein [Xanthomonas sp. SHU308]
Le0144	putative membrane protein [Pseudomonas fluorescens SBW25]
Le0172	hypothetical protein [Rhodanobacter thiooxydans]
Le0178	NA
Le0190	GCN5 family acetyltransferase [Singulisphaera acidiphila]
Le0194	NA
Le0213	conserved hypothetical protein [Azotobacter vinelandii]
Le0215	hypothetical protein [Pseudomonas aeruginosa]
Le0239	pilus assembly protein [Stenotrophomonas maltophilia]
Le0273	NA
Le0284	pilus assembly protein [Stenotrophomonas maltophilia]
Le0297	peptide transporter [Singularimonas variicoloris]
Le0303	hypothetical protein [Stenotrophomonas maltophilia]
Le0307	unnamed protein product [Photorhabdus luminescens subsp. laumondii TTO1]
Le0311	glycosyl hydrolase [Stenotrophomonas maltophilia]
Le0315	DeoR family transcriptional regulator [Xanthomonas sp. SHU199]
Le0345	NA
Le0387	pyrroloquinoline quinone biosynthesis protein PqqB [Xanthomonas vesicatoria]
Le0388	pyrroloquinoline quinone biosynthesis protein PqqC [Xanthomonas campestris]
Le0389	coenzyme PQQ biosynthesis protein D [Xanthomonas sp. SHU308]
Le0525	Alkyl hydroperoxide reductase AhpD [Wohlfahrtiimonas chitiniclastica]
Le0533	protein of unknown function DUF6 transmembrane [Haliangium ochraceum DSM 14365]
Le0612	hypothetical protein [Stenotrophomonas maltophilia]
Le0622	sugar transporter [Burkholderia pyrrocinia]
Le0623	hypothetical protein [Dyella ginsengisoli]
Le0627	hypothetical protein [Variovorax paradoxus]
Le0633	NA
Le0661	hypothetical protein [Coralloccoccus coralloides]
Le0662	hypothetical protein [Coralloccoccus coralloides]

Le0695	hypothetical protein [Achromobacter piechaudii]
Le0748	aspartyl-asparaginyl beta-hydroxylase [Xanthomonas axonopodis]
Le0753	hypothetical protein [Alcanivorax dieselolei]
Le0754	NA
Le0797	CAAX amino terminal protease [Burkholderia sp. CCGE1002]
Le0840	Acetyltransferase, GNAT family protein [Polymorphum gilvum SL003B-26A1]
Le0866	hypothetical protein [Arhodomonas aquaeolei]
Le0873	NA
Le0915	Beta-lactamase [Azospirillum lipoferum]
Le0923	hypothetical protein [Pseudomonas geniculata]
Le0926	hypothetical protein [Stenotrophomonas maltophilia]
Le0927	lanthionine synthetase C-like protein [Lysobacter sp. ATCC 53042]
Le0969	GCN5-related N-acetyltransferase [Pseudomonas mendocina NK-01]
Le0982	NA
Le1035	acetyltransferase [Leptolyngbya sp. PCC 7375]
Le1036	integral membrane-like protein [Pseudoxanthomonas suwonensis 11-1]
Le1044	NA
Le1066	NA
Le1071	hypothetical protein [Pseudomonas mandelii]
Le1072	aerobic-type carbon monoxide dehydrogenase, large subunit CoxL/CutL-like protein [Pseudomonas sp. GM79]
Le1073	(2Fe-2S)-binding protein [Pseudomonas fluorescens]
Le1074	XshC-Cox1-family protein [Stenotrophomonas maltophilia]
Le1075	CTP: molybdopterin cytidylyltransferase [Stenotrophomonas maltophilia]
Le1077	NA
Le1079	tetracenomycin polyketide synthesis O-methyltransferase TemP [Streptomyces himastatinicus ATCC 53653]
Le1083	Lysine exporter protein (LYSE/YGGA) [Ralstonia pickettii 12D]
Le1092	propeptide, PepSY and peptidase M4 [Nitrobacter sp. Nb-311A]
Le1093	NA
Le1097	D-alanyl-D-alanine dipeptidase [Rhodanobacter sp. 2APBS1]
Le1100	transglutaminase [Pseudoxanthomonas sp. GW2]
Le1111	oxidoreductase [Xanthomonas campestris]
Le1112	putative ferredoxin protein [Xanthomonas albilineans GPE PC73]
Le1116	Uncharacterized metalloprotease [Stenotrophomonas maltophilia]
Le1120	hypothetical protein [Leeia oryzae]
Le1122	hypothetical protein [Leeia oryzae]
Le1124	NA
Le1161	putative transcriptional regulator [Xanthomonas sacchari]
Le1164	hypothetical protein [Burkholderia sp.]
Le1168	putative RNA polymerase sigma factor [Xanthomonas sacchari]
Le1181	conserved hypothetical protein [Xanthomonas campestris]
Le1195	major facilitator superfamily protein [Rhodanobacter fulvus Jip2]

Le1196	NA
Le1198	family 2 glycosyl transferase [<i>Granulicella mallensis</i>]
Le1199	hypothetical protein [<i>Xanthomonas axonopodis</i>]
Le1202	NA
Le1204	NA
Le1205	N-acetylmuramyl-L-alanine amidase, negative regulator of AmpC, AmpD [<i>Niastella koreensis</i>]
Le1206	NA
Le1208	NA
Le1210	3-carboxymuconate cyclase [<i>Pseudomonas</i> sp. GM17]
Le1222	hypothetical protein [<i>Rhodanobacter spathiphylli</i>]
Le1273	ABC transporter substrate-binding protein [<i>Stenotrophomonas maltophilia</i>]
Le1305	conserved hypothetical protein [<i>Methylococcus capsulatus</i>]
Le1319	NA
Le1321	hypothetical protein HMPREF0995_05895, partial [<i>Lachnospiraceae</i> bacterium 7_1_58FAA]
Le1372	cupin domain-containing protein [<i>Rhodanobacter</i> sp. 116-2]
Le1373	ArsR family transcriptional regulator [<i>Burkholderia</i> sp. JPY251]
Le1378	NA
Le1379	hypothetical protein [<i>Variovorax paradoxus</i>]
Le1383	putative esterase of the alpha-beta hydrolase superfamily [<i>Deinococcus peraridilitoris</i> DSM 19664]
Le1388	putative protease [<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i> PCC21]
Le1391	4-oxalocrotonate tautomerase [<i>Achromobacter piechaudii</i>]
Le1394	hypothetical protein [<i>Rudaea cellulositytica</i>]
Le1421	GCN5-related N-acetyltransferase [<i>Thioalkalivibrio sulfidophilus</i> HL-EbGr7]
Le1422	NA
Le1427	hypothetical protein [<i>Rudaea cellulositytica</i>]
Le1458	YadA domain protein [<i>Burkholderia ambifaria</i> MEX-5]
Le1463	NA
Le1495	hypothetical protein [<i>Streptomyces somaliensis</i>]
Le1498	hypothetical protein [<i>Streptomyces</i> sp. FxanaD5]
Le1500	NA
Le1501	Protein of unknown function (DUF3526) [<i>Novosphingobium</i> sp. AP12]
Le1504	protein with peptidoglycan-binding domain [<i>Psychroflexus torquis</i>]
Le1505	hypothetical protein [<i>Myxococcus</i> sp. (contaminant ex DSM 436), A176_1024]
Le1540	hypothetical protein CATMIT_01824, partial [<i>Catenibacterium mitsuokai</i> DSM 15897]
Le1544	NA
Le1558	methyltransferase [<i>Stenotrophomonas maltophilia</i>]
Le1566	putative glycosyl transferase PsII [<i>Pseudomonas fluorescens</i> A506]
Le1567	NA
Le1568	arabinose efflux permease family protein [<i>Phyllobacterium</i> sp. YR531]
Le1570	NA
Le1574	unknown [<i>Lysobacter enzymogenes</i>]
Le1575	unknown [<i>Lysobacter enzymogenes</i>]

Le1577	SctL [Lysobacter enzymogenes]
Le1578	NA
Le1579	unknown [Lysobacter enzymogenes]
Le1581	unknown [Lysobacter enzymogenes]
Le1585	PhaA [Lysobacter enzymogenes]
Le1589	NA
Le1591	type III secretion protein, HrpO family [Burkholderiales bacterium JOSHI_001]
Le1594	NA
Le1595	hypothetical protein [gamma proteobacterium]
Le1614	NA
Le1615	NA
Le1652	relaxation protein [Xanthomonas campestris]
Le1675	transmembrane anti-sigma factor [Pseudomonas fluorescens]
Le1680	putative protease with the C-terminal PDZ domain [Pseudoxanthomonas spadix BD-a59]
Le1682	probable glucans biosynthesis protein d precursor [Xanthomonas albilineans GPE PC73]
Le1689	hypothetical protein [Dyella ginsengisoli]
Le1691	hypothetical protein [Dyella ginsengisoli]
Le1694	hypothetical protein [Rudaea cellulositytica]
Le1695	hypothetical protein [Rudaea cellulositytica]
Le1702	NA
Le1719	NA
Le1723	NA
Le1724	hypothetical protein [Stenotrophomonas maltophilia]
Le1740	pyridine nucleotide-disulfide oxidoreductase [Pseudoxanthomonas suwonensis]
Le1750	NA
Le1753	hypothetical protein [Enterobacter cloacae]
Le1760	peptidase C39 [Burkholderia pyrrocinia]
Le1770	NA
Le1792	putative metal-dependent membrane protease [Xanthomonas axonopodis pv. citrumelo F1]
Le1806	2, 5-diketo-D-gluconic acid reductase [Variovorax paradoxus]
Le1810	hypothetical protein [Delftia acidovorans]
Le1822	NA
Le1823	NA
Le1837	NA
Le1869	putative integron gene cassette protein [uncultured bacterium]
Le1870	streptomycin 3"-phosphotransferase [Achromobacter xylosoxidans C54]
Le1922	NA
Le1965	endoribonuclease L-PSP [Burkholderia multivorans]
Le1966	short chain fatty acid transporter [Stenotrophomonas maltophilia]
Le1982	NA
Le1991	heat shock protein Hsp70 [Xanthomonas gardneri]
Le1999	hypothetical protein [Xanthomonas vesicatoria]
Le2006	conserved hypothetical protein [Xanthomonas campestris]

Le2013	NA
Le2014	NA
Le2015	NA
Le2017	NA
Le2029	RDD family protein [Cesiribacter andamanensis]
Le2048	kanamycin nucleotidyltransferase, partial [Catenibacterium mitsuokai DSM 15897]
Le2071	NA
Le2109	NA
Le2143	hypothetical protein [uncultured bacterium, ACD_23C00190G0001]
Le2145	NA
Le2156	GCN5 family acetyltransferase [Rhodanobacter fulvus]
Le2179	conserved hypothetical protein [Ralstonia syzygii R24]
Le2180	hypothetical protein [Dyella ginsengisoli]
Le2185	NA
Le2196	membrane protein [Xanthomonas sacchari]
Le2198	NA
Le2199	hypothetical protein [Gordonia polyisoprenivorans]
Le2260	polymerase [Stenotrophomonas maltophilia]
Le2270	N-acylglucosamine 2-epimerase [Stenotrophomonas maltophilia]
Le2274	sugar hydrolase [Pseudomonas geniculata]
Le2286	conserved hypothetical protein [Stigmatella aurantiaca]
Le2288	hypothetical protein [Acidovorax citrulli]
Le2359	putative iron transport/utilization related protein [Burkholderia xenovorans LB400]
Le2407	glyoxalase/bleomycin resistance protein/dioxygenase [Rhodococcus triatomae]
Le2417	TetR family transcriptional regulator [Rhizobium leguminosarum]
Le2427	trans-2-enoyl-CoA reductase [Xanthomonas campestris]
Le2450	lanthionine synthetase [Stenotrophomonas maltophilia]
Le2521	hypothetical protein [Herbaspirillum sp. JC206]
Le2530	hypothetical protein [Stenotrophomonas maltophilia]
Le2531	hypothetical protein [Xanthomonas sacchari]
Le2540	hypothetical protein [Bifidobacterium adolescentis]
Le2552	hypothetical protein [Pseudoxanthomonas sp. GW2]
Le2572	NA
Le2616	hypothetical protein [Rudaea cellulositytica]
Le2620	NA
Le2627	hypothetical protein, partial [Xanthomonas sp. SHU199]
Le2636	conserved hypothetical exported protein [Stenotrophomonas maltophilia K279a]
Le2649	membrane protein [Stenotrophomonas maltophilia]
Le2660	hypothetical protein [Micromonospora sp.]
Le2683	glycerol kinase [Xanthomonas sp. SHU199]
Le2685	glycerol-3-phosphate dehydrogenase [Rhodanobacter sp. 115]
Le2703	beta-lytic protease [Lysobacter sp. IB-9374]
Le2716	hypothetical protein [Rudaea cellulositytica]

Le2717	Hemolysin-type calcium-binding repeat (2 copies) [Candidatus Chloracidobacterium thermophilum B]
Le2718	NA
Le2719	NA
Le2720	NA
Le2726	hypothetical protein [Variovorax sp. CF313, PMI12_00324]
Le2727	transcriptional regulator, AraC family [Variovorax paradoxus EPS]
Le2772	NA
Le2784	hypothetical protein [Rhodanobacter spathiphylli]
Le2785	hypothetical protein [Dyella ginsengisoli]
Le2790	hemolysin-type calcium-binding protein [Clostridium cellulovorans]
Le2830	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase [Xanthomonas translucens]
Le2859	NA
Le2871	endoglucanase [Lysobacter sp. IB-9374]
Le2884	conserved hypothetical protein [Variovorax paradoxus]
Le2926	lysine exporter protein [Azoarcus sp. KH32C]
Le2927	NA
Le2933	hypothetical protein [Stenotrophomonas maltophilia Ab55555, A1OC_03568]
Le2955	NA
Le2980	TetR family transcriptional regulator [Myxococcus stipitatus]
Le2997	NA
Le3002	hypothetical protein [Xanthomonas translucens]
Le3019	predicted phosphohydrolase [Hahella chejuensis]
Le3022	hypothetical protein [Xanthomonas translucens]
Le3026	hypothetical protein [Dyella japonica]
Le3046	hypothetical protein [Stenotrophomonas maltophilia Ab55555, A1OC_03423]
Le3060	cytochrome c, class I [Cupriavidus sp. HMR-1]
Le3062	(2Fe-2S)-binding protein [Ralstonia sp. AU12-08]
Le3075	Cupin 2 conserved barrel domain protein [Pseudomonas fulva]
Le3100	Rieske (2Fe-2S) domain-containing protein [Xanthomonas translucens]
Le3180	NA
Le3194	membrane protein [Achromobacter xylosoxidans]
Le3199	NA
Le3201	lysine transporter LysE [Mesorhizobium amorphae]
Le3202	hypothetical protein [Roseomonas sp. B5]
Le3205	hypothetical protein [Chryseobacterium taeanense]
Le3211	hypothetical protein [Uliginosibacterium gangwonense]
Le3226	lipolytic protein G-D-S-L [Collimonas fungivorans Ter331]
Le3232	2-methylcitrate dehydratase [Rhodanobacter sp.]
Le3286	MATE efflux family protein [Thauera sp. 63]
Le3290	bleomycin resistance protein [Rhizobium sp. 42MFCr.1]
Le3292	hypothetical protein [Dyella ginsengisoli]

Le3299	putative methyltransferase [<i>Xanthomonas campestris</i>]
Le3300	hypothetical protein [<i>Xanthomonas</i> sp. SHU199]
Le3307	inner membrane protein [<i>Xanthomonas translucens</i>]
Le3311	hypothetical protein [<i>Variovorax paradoxus</i> EPS, Varpa_1846]
Le3316	hypothetical protein [<i>Xanthomonas arboricola</i>]
Le3322	TfoX domain-containing protein [<i>Collimonas fungivorans</i>]
Le3330	glyoxalase [<i>Stenotrophomonas maltophilia</i>]
Le3333	hypothetical protein [<i>Xanthomonas translucens</i>]
Le3362	hypothetical protein [<i>Nodosilinea nodulosa</i>]
Le3387	NA
Le3393	hypothetical protein [<i>Variovorax</i> sp.]
Le3408	putative transmembrane protein [<i>Stenotrophomonas maltophilia</i>]
Le3429	NAD(P)H dehydrogenase [<i>Xanthomonas</i> sp. SHU199]
Le3452	putative secreted protein [<i>Stenotrophomonas maltophilia</i> D457]
Le3466	lactoylglutathione lyase [<i>Dyella japonica</i>]
Le3473	phospholipase C, phosphocholine-specific [<i>Pseudomonas aeruginosa</i>]
Le3474	hypothetical protein [<i>Klebsiella oxytoca</i>]
Le3475	hypothetical protein [<i>Proteus penneri</i>]
Le3476	protein kinase/helix-hairpin-helix DNA-binding domain-containing protein [<i>Salinisphaera shabanensis</i>]
Le3480	conserved hypothetical protein [<i>Xanthomonas campestris</i>]
Le3483	hypothetical protein [<i>Xanthomonas axonopodis</i>]
Le3491	NA
Le3508	NA
Le3518	hypothetical protein [Acetobacteraceae bacterium]
Le3534	protein of unknown function DUF336 [Candidatus <i>Solibacter usitatus</i> Ellin6076]
Le3541	cytochrome C551 [<i>Pseudoxanthomonas</i> sp. GW2]
Le3542	hypothetical protein [<i>Xanthomonas sacchari</i>]
Le3554	periplasmic glucan biosynthesis protein, MdoG [<i>Roseomonas cervicalis</i> ATCC 49957]
Le3555	Glucans biosynthesis glucosyltransferase H [<i>Xanthomonas campestris</i>]
Le3595	SMP-30/gluconolactonase/LRE-like region-containing protein [<i>Pseudoxanthomonas suwonensis</i>]
Le3616	NA
Le3668	NA
Le3689	hypothetical protein [<i>Gemmata obscuriglobus</i>]
Le3691	hypothetical protein [<i>Pseudomonas mendocina</i>]
Le3704	hypothetical protein [<i>Xanthomonas axonopodis</i>]
Le3714	hypothetical protein [<i>Dyella japonica</i>]
Le3732	NA
Le3757	hypothetical protein [<i>Xanthomonas</i> sp. SHU199]
Le3760	membrane protein [<i>Xanthomonas campestris</i>]
Le3811	hemin storage protein [<i>Stenotrophomonas maltophilia</i>]
Le3812	alpha-1, 2-mannosidase [<i>Rhodanobacter thiooxydans</i> LCS2]
Le3814	NA

Le3815	NA
Le3847	hypothetical protein [Xanthomonas sacchari]
Le3858	putative phospholipase c precursor protein [Xanthomonas albilineans]
Le3884	NA
Le3904	hypothetical protein [Janthinobacterium sp. CG3]
Le3905	MFS transporter [Serratia marcescens]
Le3915	hypothetical protein [Rubrivivax gelatinosus]
Le3917	endonuclease [Stenotrophomonas maltophilia]
Le3925	NA
Le3940	NA
Le3944	hypothetical protein [Candidatus Solibacter]
Le3991	hypothetical protein [Pseudoxanthomonas spadix]
Le4025	NA
Le4030	NA
Le4067	membrane protein [Pseudomonas chlororaphis]
Le4071	putative transmembrane protein [Stenotrophomonas maltophilia]
Le4074	lysine exporter protein LysE/YggA [Pseudomonas sp. CF149]
Le4102	hypothetical protein [Xanthomonas sp. SHU199]
Le4115	NA
Le4136	conserved hypothetical protein [Xanthomonas citri]
Le4138	hypothetical protein [Xanthomonas fragariae]
Le4141	putative transcriptional regulator [Mesorhizobium metallidurans]
Le4163	NA
Le4180	hypothetical protein [Xanthomonas sacchari]
Le4187	NA
Le4199	putative membrane protein [Mesorhizobium amorphae]
Le4224	hypothetical protein [Bifidobacterium adolescentis]
Le4225	hypothetical protein BIFADO_01105, partial [Bifidobacterium adolescentis L2-32]
Le4231	NA
Le4252	hypothetical protein [Methylobacterium radiotolerans]
Le4258	hypothetical protein [Stenotrophomonas maltophilia]
Le4260	sugar ABC transporter substrate-binding protein [Xanthomonas translucens]
Le4261	binding-protein-dependent transport systems inner membrane component
Le4262	sugar ABC transporter permease [Stenotrophomonas maltophilia]
Le4263	hypothetical protein [Xanthomonas translucens]
Le4272	NA
Le4275	MFS transporter [Dyella ginsengisoli]
Le4301	NA
Le4302	NA
Le4306	NAD(P)H dehydrogenase [Pseudomonas aeruginosa]
Le4323	Glyoxalase/bleomycin resistance protein/dioxygenase [Pseudoxanthomonas suwonensis]
Le4332	hypothetical protein [Stenotrophomonas maltophilia]
Le4337	NA

Le4383	hypothetical protein [Sphingobium sp.]
Le4386	membrane protein [Sphingobium lactosutens DS20]
Le4387	membrane protein [Stenotrophomonas maltophilia]
Le4388	hypothetical protein [alpha proteobacterium LLX12A]
Le4421	hypothetical protein [Xanthomonas oryzae]
Le4422	copper resistance protein M [Xanthomonas alfalfae subsp. citrumelonis]
Le4427	NA
Le4432	FAD-binding 9 siderophore-interacting domain-containing protein
Le4467	cycloisomaltooligosaccharide glucanotransferase [Xanthomonas sp. SHU199]
Le4474	kanamycin nucleotidyltransferase, partial [Catenibacterium mitsuokai DSM 15897]
Le4527	putative uncharacterized protein [Burkholderia sp. RPE64]
Le4557	chemotaxis protein CheW [Pseudomonas fluorescens]
Le4558	putative chemotaxis-related methyltransferase [Pseudomonas fluorescens Pf0-1]
Le4559	chemotaxis protein CheW [Herbaspirillum frisingense]
Le4572	lanthionine synthetase C-like protein [Lysobacter sp. ATCC 53042]
Le4573	hypothetical protein [Lysobacter sp. ATCC 53042]
Le4588	beta-lactamase domain-containing protein [Lysobacter sp. ATCC 53042]
Le4595	putative Mg(2+) transporter [Lysobacter sp. ATCC 53042]
Le4598	major facilitator family transporter [Lysobacter sp. ATCC 53042]
Le4604	cytochrome-c peroxidase [Lysobacter sp. ATCC 53042]
Le4640	hydrolase [Burkholderia pyrrocinia]
Le4641	hypothetical protein [Rudaea cellulolytica]
Le4644	hypothetical protein [Xanthomonas sp. SHU199]
Le4645	hypothetical protein [Rhodanobacter fulvus]
Le4648	transcriptional regulator [Fluoribacter dumoffii]
Le4650	hypothetical protein [uncultured bacterium, ACD_54C00931G0002]
Le4651	hypothetical protein [Xanthomonas sp. SHU199]
Le4683	NA
Le4696	NA
Le4697	NA
Le4734	beta-mannosidase [Stenotrophomonas maltophilia]
Le4738	alpha-1 2-mannosidase [Xanthomonas axonopodis]
Le4742	copper homeostasis protein CutC [Stenotrophomonas maltophilia]
Le4752	NA
Le4755	NA
Le4758	hypothetical protein [Lamprocystis purpurea]
Le4759	glycosyl transferase [Vibrio breoganii]
Le4791	NA
Le4792	NA
Le4796	NA
Le4798	putative exported protein [Xanthomonas translucens]
Le4800	hypothetical protein [Achromobacter xylosoxidans]
Le4801	hypothetical protein [Acidovorax sp. MR-S7, AVS7_01837]

Le4802	hypothetical protein [Uliginosibacterium gangwonense]
Le4803	NA
Le4804	NAD dependent epimerase/dehydratase [Pseudomonas sp. GM80]
Le4805	putative thiol-disulfide oxidoreductase DCC [Burkholderia gladioli]
Le4816	NA
Le4832	hypothetical protein [Rudaea cellulositytica]
Le4857	hypothetical protein [Methylomonas methanica]
Le4860	hypothetical protein [Stenotrophomonas maltophilia Ab55555, A1OC_03207]
Le4876	hypothetical protein [Xanthomonas vesicatoria]
Le4888	alkane 1-monoxygenase [Xanthomonas sacchari]
Le4891	NA
Le4909	NA
Le4911	NA
Le4916	NA
Le4918	hypothetical protein [Cellvibrio sp.]
Le4933	hypothetical protein [Halomonas anticariensis]
Le4945	hypothetical protein [Xanthomonas sacchari]
Le4951	hypothetical protein [Rhodanobacter sp.]
Le4968	glyoxalase [Kaistia granuli]
Le4977	hypothetical protein [Variovorax sp.]
Le4978	hypothetical protein [Acetobacter tropicalis]
Le4980	carbohydrate-binding CenC domain protein [Conexibacter woesei]
Le4981	NA
Le4983	alpha/beta hydrolase fold protein [Pseudoxanthomonas suwonensis]
Le5000	protein of unknown function DUF1470 [Granulicella tundricola MP5ACTX9]
Le5013	NA
Le5022	hypothetical protein [Xanthomonas translucens]
Le5025	hypothetical protein [Enterococcus moraviensis]
Le5040	NA
Le5047	putative membrane protein [Xanthomonas translucens]
Le5053	hypothetical protein [Xanthomonas sp. SHU199]
Le5079	NA
Le5104	putative transcriptional regulator, MarR family
Le5141	NA
Le5159	NA
Le5160	NA
Le5161	NA
Le5167	NA
Le5172	NA
Le5173	NA
Le5202	hypothetical protein, partial [Xanthomonas perforans]
Le5236	NA
Le5260	NA

1

Table S2. Distribution of Le4759 and Le0100 in non-*Lysobacter* bacterial genomes

Organisms	LuxR	LuxI	Le4759	Le0100
<i>Luteimonas aquatica</i> RIB1-20	WP_242108327.1	none	WP_242113092.1	none
<i>Arenimonas composti</i> TR7-09	none	none	WP_026816606.1	none
<i>Thermomonas alba</i> SYSU G04536	none	none	WP_240125151.1	none
<i>Halomonas aerodenitrificans</i> MCCC 1A11058	none	none	WP_234255712.1	none
<i>Tahibacter caeni</i> BUT-6	WP_257385461.1	none	none	WP_257386727.1
<i>Pseudomonas fluorescence</i> 2P24	AAT42219.1	AAT42217.1	none	none
<i>Pectobacterium carotovorum</i> PccS1	WP_181848026.1	WP_015842234.1	none	none
<i>Pseudomonas arenosa</i> CAU 1598	WP_225444625.1	WP_192030098.1	none	none
<i>Aquimonas voraii</i> DSM 16957	WP_176764180.1	WP_176764181.1	WP_091242174.1	none
<i>Nitrospira lacus</i> APG3	WP_004180566.1	WP_004180210.1	WP_004180885.1	none
<i>Nitrospira briensis</i> C-128	WP_025041731.1	WP_025041701.1	WP_025040903.1	none
<i>Candidatus Nitrotoga</i> AM1P	WP_173052046.1	WP_242007018.1	WP_173052373.1	none
<i>Nitrosovibrio tenuis</i> Nv1	WP_090826092.1	WP_090826837.1	WP_090827938.1	none
<i>Nitrospira multiformis</i> N11	WP_074630300.1	WP_218124298.1	WP_074630447.1	none
<i>Ahniella affigens</i> D13	WP_106891580.1	WP_106891581.1	WP_170113451.1	none
<i>Paraburkholderia dipogonis</i> ICMP 19430	WP_106300542.1	WP_134460123.1	WP_134457033.1	none

1

Table S3. Strains and plasmids used in this study

Strains and plasmids	Characteristics ^a	Source
Wild-type strains		
<i>Lysobacter enzymogenes</i> OH11	Wild type, Km ^R	(1)
<i>Pseudomonas fluorescence</i> 2P24	Wild type	(2)
<i>Pectobacterium carotovorum</i> PccS1	Wild type, Rif ^R	
JZA1	<i>Agrobacterium tumefaciens</i> carrying an AHL reporter plasmid with a traG::lacZ fusion, Sp ^R , Gm ^R , Tc ^R	(3)
<i>Escherichia coli</i>		
DH5α	Host strain for molecular cloning	(4)
XL1-Blue MRF ⁻ Kan	Host for bacterial two-hybrid assay	(4)
BL21(DE3)	Host strain for protein expression, Km ^R	(4)
JMP	JM109 with a <i>P_{BAD}::pcol</i> fragment insertion on chromosome	Laboratory stock
In-frame deletion mutants		
Δ <i>pcol</i>	In-frame deletion of <i>pcol</i> in strain 2P24, Km ^R	(2)
Overexpression strains		
2P24 (<i>gfp</i>)	2P24 harbouring plasmid pBBR- <i>gfp</i> , Gm ^R	This study
2P24 (<i>Le4759</i>)	2P24 harbouring plasmid pBBR- <i>Le4759</i> , Gm ^R	This study
JMP(<i>gfp</i>)	JMP harbouring plasmid pBBR- <i>gfp</i> , Gm ^R	This study
JMP(<i>Le4759</i>)	JMP harbouring plasmid pBBR- <i>Le4759</i> , Gm ^R	This study
JMP(EV)	JMP harbouring pBBR empty plasmid, Gm ^R	This study
JMP(<i>momL</i>)	JMP harbouring plasmid pBBR- <i>momL</i> , Gm ^R	This study
PccS1 (<i>Le4759</i>)	PccS1 harbouring plasmid pBBR- <i>Le4759</i> , Gm ^R , Rif ^R	This study
PccS1 (<i>gfp</i>)	PccS1 harbouring plasmid pBBR- <i>gfp</i> , Gm ^R , Rif ^R	This study
PccS1 (<i>Le4759^{R70L}</i>)	PccS1 harbouring plasmid pBBR- <i>Le4759^{R70L}</i> , Gm ^R , Rif ^R	This study
PccS1 (<i>Le4759^{R84H}</i>)	PccS1 harbouring plasmid pBBR- <i>Le4759^{R84H}</i> , Gm ^R , Rif ^R	This study
PccS1 (<i>Le4759^{I-200}</i>)	PccS1 harbouring plasmid pBBR- <i>Le4759^{I-200}</i> , Gm ^R , Rif ^R	This study
PccS1 (<i>Le4759^{I2-383}</i>)	PccS1 harbouring plasmid pBBR- <i>Le4759^{I2-383}</i> , Gm ^R , Rif ^R	This study
Δ <i>pcol</i> (<i>gfp</i>)	Δ <i>pcol</i> harbouring plasmid pBBR- <i>gfp</i> , Gm ^R	This study
Δ <i>pcol</i> (<i>Le4759</i>)	Δ <i>pcol</i> harbouring plasmid pBBR- <i>Le4759</i> , Gm ^R	This study
Δ <i>pcol</i> (<i>momL</i>)	Δ <i>pcol</i> harbouring plasmid pBBR- <i>momL</i> , Gm ^R	This study
DH5α (<i>carl</i> & <i>Le4759</i>)	DH5α harbouring plasmid pBBR- <i>Le4759</i> and pUCP26- <i>carl</i> Tc ^R , Gm ^R	This study
DH5α (<i>carl</i> & <i>gfp</i>)	DH5α harbouring plasmid pBBR- <i>gfp</i> and pUCP26- <i>carl</i> Tc ^R , Gm ^R	This study
DH5α (<i>carl</i> & EV)	DH5α harbouring pBBR empty plasmid and pUCP26- <i>carl</i> Tc ^R , Gm ^R	This study
DH5α (<i>lbsI</i> & <i>Le4759</i>)	DH5α harbouring plasmid pBBR- <i>Le4759</i> and pUCP26- <i>lbsI</i> Tc ^R , Gm ^R	This study
DH5α (<i>lbsI</i> & <i>gfp</i>)	DH5α harbouring plasmid pBBR- <i>gfp</i> and pUCP26- <i>lbsI</i> Tc ^R , Gm ^R	This study
DH5α (<i>lbsI</i> & EV)	DH5α harbouring pBBR empty plasmid and pUCP26- <i>lbsI</i> Tc ^R , Gm ^R	This study
Plasmids		
pBBR-MCS5	Broad-host-vector with a P _{lac} promoter, Gm ^R	(5)
pTRG	The plasmid used for protein expression in bacterial two-hybridization assay, Tc ^R	(6)
pBT	The plasmid used for protein expression in bacterial two-hybridization assay, Chlo ^R	(6)
pBT- <i>Le4759</i>	pBT with the coding region of <i>Le4759</i> from strain OH11, Chl ^R	This study
pBT- <i>gacS</i>	pBT containing the coding region of <i>gacS</i>	(4)
pTRG- <i>pcol</i>	pTRG with the coding region of <i>pcol</i> , Tc ^R	This study
pTRG- <i>lbsI</i>	pTRG with the coding region of <i>lbsI</i> , Tc ^R	This study
pTRG- <i>carl</i>	pTRG containing the coding region of <i>carl</i> , Tc ^R	This study
pTRG- <i>gacS</i>	pTRG containing the coding region of <i>gacS</i> , Tc ^R	This study
pBBR- <i>gfp</i>	pBBR-MCS5 with the coding region of <i>gfp</i> , Gm ^R	This study
pBBR- <i>pcol</i>	pBBR-MCS5 with the coding region of <i>pcol</i> , Gm ^R	This study
pBBR- <i>Le4759</i>	pBBR-MCS5 with the coding region of <i>Le4759</i> , Gm ^R	This study
pBBR- <i>Le4759^{R70L}</i>	pBBR-MCS5 with the coding region of <i>Le4759^{R70L}</i> , Gm ^R	This study
pBBR- <i>Le4759^{R84H}</i>	pBBR-MCS5 with the coding region of <i>Le4759^{R84H}</i> , Gm ^R	This study
pBBR- <i>Le4759^{I-200}</i>	pBBR-MCS5 with the coding region of <i>Le4759^{I-200}</i> , Gm ^R	This study
pBBR- <i>Le4759^{I2-383}</i>	pBBR-MCS5 with the coding region of <i>Le4759^{I2-383}</i> , Gm ^R	This study
pBBR- <i>momL</i>	pBBR-MCS5 with the coding region of <i>momL</i> , Gm ^R	This study
pUCP26- <i>pcol</i>	pUCP26 with the coding region of <i>pcol</i> , Gm ^R	This study
pUCP26- <i>carl</i>	pUCP26 with the coding region of <i>carl</i> , Gm ^R	This study
pUCP26- <i>lbsI</i>	pUCP26 with the coding region of <i>lbsI</i> , Gm ^R	This study

2 ^aKm^R, Gm^R Amp^R, Tc^R, Chlo^R, Sp^R, kanamycin, gentamicin, ampicillin, tetracycline, chloramphenicol, spectinomycin resistance,
3 respectively.

4

5

Table S4. Primers used in this study

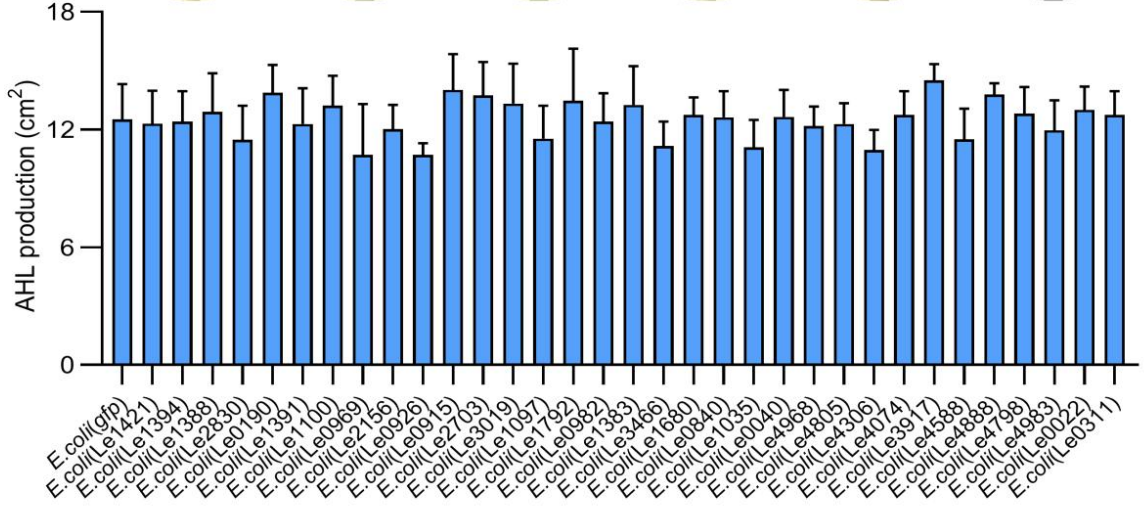
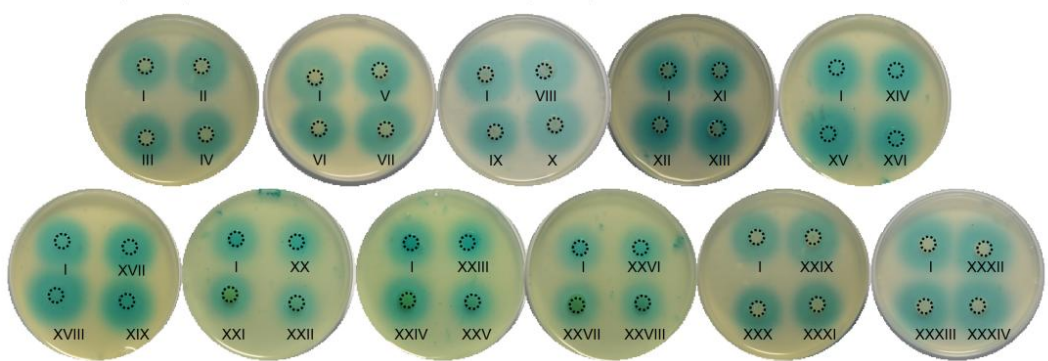
Primer	Sequence (5'-3') ^a	Purpose
Overexpression		
<i>Le4759</i> -F	AGGGAACAAAAGCTGGGTACCgATGCGCATCTTGTTCCTGACCG (<i>KpnI</i>)	To amplify a 1257-bp fragment of <i>Le4759</i> fused with a FLAG tag
<i>Le4759</i> -FLAG-R	CGCGGTGGCGGCCGCTCTAGATTACTTATCGTCGTCATCCTTGTAAATCCTCCGGCGTGCACAACAGGC (<i>XbaII</i>)	
<i>momL</i> -F	CCCAAGCTTgAtgaatatgaaaaagctactgttac (<i>HindIII</i>)	To amplify a 1377-bp fragment of <i>momL</i>
<i>momL</i> -R	TGCTCTAGAttattgtaaagtgggtgcctgg(<i>XbaII</i>)	
<i>Le4759</i> ²¹²⁻³⁸³ -F	CGCGGTGGCGGCCGCTCTAGATTACTTATCGTCGTCATCCTTGTAAATCGCCGTGAGCACGACGTGGATCTT(<i>KpnI</i>)	To amplify a 516-bp downstream homologue of <i>Le4759</i> ²¹²⁻³⁸³
<i>Le4759</i> ¹⁻²⁰⁰ -FLAG-R	CGCGGTGGCGGCCGCTCTAGATCACTTATCGTCGTCATCCTTGTAAATCCACGCTGGCGGCAGCACGGCGAAG (<i>XbaII</i>)	To amplify a 600-bp upstream homologue of <i>Le4759</i> ¹⁻²⁰⁰ fused with a FLAG tag
<i>Le4759</i> ^{R70L} -F	TCCGGGTGGTGCtGGTCAAGACC	To amplify a 1061-bp downstream homologue of <i>Le4759</i> ^{R70L}
<i>Le4759</i> ^{R70L} -R	GGTCTTGACCAGCACCACCCGGA	To amplify a 219-bp upstream homologue of <i>Le4759</i> ^{R70L}
<i>Le4759</i> ^{R84H} -F	GCTTCCTCAAGCaCACCTGGAC	To amplify a 1019-bp downstream homologue of <i>Le4759</i> ^{R84H}
<i>Le4759</i> ^{R84H} -R	GTCCAGGGTGTGCTTGAGGAAGC	To amplify a 216-bp upstream homologue of <i>Le4759</i> ^{R84H}
<i>gfp</i> -FLAG-F	CCCAAGCTTgATGAGTAAAGGTGAAGAAGTGT (<i>HindIII</i>)	To amplify a 717-bp fragment upstream of <i>gfp</i> fused with a FLAG tag
<i>gfp</i> -FLAG-R	TGCTCTAGATCACTTATCGTCGTCATCCTTGTAAATCTTTGTAGAGTTCATCCATG (<i>XbaI</i>)	
Protein expression and Pull-down assays		
pET30a- <i>pcol</i> -F	GGAATCCATATGAAATACTTAATAGACAAAACGCGAG(<i>NdeI</i>)	To amplify a 576-bp fragment of <i>pcol</i>
pET30a- <i>pcol</i> -R	CCCAAGCTTACCACCGTTGACGGCGTCCAACG (<i>HindIII</i>)	
pET30a- <i>carI</i> -F	ACTTAAAGAAGGAGATATACATATGATGTTAGAGATATTTGATG(<i>NdeI</i>)	To amplify a 651-bp fragment of <i>carI</i>
pET30a- <i>carI</i> -R	GGTGCTCGAGTGCGGCCGCAAGCTTAGCCTGTACAATAGCGGCAG (<i>HindIII</i>)	
pET30a- <i>lbsI</i> -F	ACTTAAAGAAGGAGATATACATATGATGACCCGATTGCCATCGGC(<i>NdeI</i>)	To amplify a 618-bp fragment of <i>lbsI</i>
pET30a- <i>lbsI</i> -R	GGTGCTCGAGTGCGGCCGCAAGCTTGGCTGCGCGGGCGATGTCGAGT (<i>HindIII</i>)	
pET30a- <i>Le4759</i> -F	ACTTAAAGAAGGAGATATACATATGATGCGCATCTTGTTCCTGACCG(<i>NdeI</i>)	To amplify a 1257-bp fragment of <i>Le4759</i>
pET30a- <i>Le4759</i> -R	GGTGCTCGAGTGCGGCCGCAAGCTTCTCCGGCGTGCACAACAGGC (<i>HindIII</i>)	
Co-IP assays		
<i>Le4759</i> -F	AGGGAACAAAAGCTGGGTACCgATGCGCATCTTGTTCCTGACCG (<i>KpnI</i>)	To amplify a 1257-bp fragment of <i>Le4759</i> fused with a FLAG tag
<i>Le4759</i> -FLAG-R	CGCGGTGGCGGCCGCTCTAGATTACTTATCGTCGTCATCCTTGTAAATCCTCCGGCGTGCACAACAGGC (<i>XbaII</i>)	
<i>pcol</i> -His-F	CTATGACCATGATTACGAATTCgATGAAATACTTAATAGACAAAACGCG (<i>EcoRI</i>)	To amplify a 576-bp fragment of <i>pcol</i> fused with a His tag
<i>pcol</i> -His-R	GCAGGTCGACTCTAGAGGATCCTTAATGATGATGATGATGGTGACCACCGTTGACGGCGTCCAACG (<i>BamHI</i>)	
<i>carI</i> -His-F	CTATGACCATGATTACGAATTCgATGTTAGAGATATTTGATG	To amplify a 651-bp fragment of <i>CarI</i> fused with a His tag
<i>carI</i> -His-R	GCAGGTCGACTCTAGAGGATCCTTAATGATGATGATGATGGTGAGCCTGTACAATAGCGGCAG	
<i>lbsI</i> -His-F	CTATGACCATGATTACGAATTCgATGACCCGATTGCCATCGGC	To amplify a 618-bp fragment of <i>LbsI</i> fused with a His tag
<i>lbsI</i> -His-R	CAGGTCGACTCTAGAGGATCCTCAATGATGATGATGATGGTGGGCTGCGCGGGCGATGTCGAGT	
<i>gfp</i> -FLAG-F	CCCAAGCTTgATGAGTAAAGGTGAAGAAGTGT (<i>HindIII</i>)	To amplify a 717-bp fragment of <i>gfp</i> fused with a FLAG tag
<i>gfp</i> -FLAG-R	TGCTCTAGATCACTTATCGTCGTCATCCTTGTAAATCTTTGTAGAGTTCATCCATG (<i>XbaI</i>)	
Bacterial two-hybrid assays		
pBT- <i>Le4759</i> -F	TGGCGCGGCCGATCGAATTCATGCGCATCTTGTTCCTGAC (<i>EcoRI</i>)	To amplify a 1257-bp fragment of <i>Le4759</i> and cloned into pBT
pBT- <i>Le4759</i> -R	AATTAATTAACCTCGAGGATCCTTACTCCGGCGTGCACAACAGG (<i>BamHI</i>)	

pTRG- <i>pcol</i> -F	AAACCAGAGGCGGCC <u>GGATCC</u> GATGAAATACTTAATA (<i>Bam</i> HI)	To amplify a 576-bp fragment of <i>pcol</i> and cloned into pTRG
pTRG- <i>pcol</i> -R	GCGCCAGCTCAGACTGAATTCCTTAACCACCGTTGACGGC (<i>Eco</i> RI)	
pTRG- <i>carI</i> -F	AAACCAGAGGCGGCC <u>GGATCC</u> ATGTTAGAGATATTGATG (<i>Bam</i> HI)	To amplify a 651-bp fragment of <i>carI</i> and cloned into pTRG
pTRG- <i>carI</i> -R	GCGCCAGCTCAGACTGAATTCCTAAGCCTGTACAATAGCGG (<i>Eco</i> RI)	
pTRG- <i>lbsI</i> -F	AAACCAGAGGCGGCC <u>GGATCC</u> ATGACCCGTATTGCCATCGG (<i>Bam</i> HI)	To amplify a 618-bp fragment of <i>lbsI</i> and cloned into pTRG
pTRG- <i>lbsI</i> -R	GCGCCAGCTCAGACTGAATTCCTCAGGCTGCGCGGGCGATGTC (<i>Eco</i> RI)	

1 ^aRestricted digestion enzyme site was underlined.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

I: <i>E. coli</i> (<i>gfp</i>)	II: <i>E. coli</i> (Le1421)	III: <i>E. coli</i> (Le1394)	IV: <i>E. coli</i> (Le1388)	V: <i>E. coli</i> (Le2830)
VI: <i>E. coli</i> (Le0190)	VII: <i>E. coli</i> (Le1391)	VIII: <i>E. coli</i> (Le1100)	IX: <i>E. coli</i> (Le0969)	X: <i>E. coli</i> (Le2156)
XI: <i>E. coli</i> (Le0926)	XII: <i>E. coli</i> (Le0915)	XIII: <i>E. coli</i> (Le2703)	XIV: <i>E. coli</i> (Le3019)	XV: <i>E. coli</i> (Le1097)
XVI: <i>E. coli</i> (Le1792)	XVII: <i>E. coli</i> (Le0982)	XVIII: <i>E. coli</i> (Le1383)	XIX: <i>E. coli</i> (Le3466)	XX: <i>E. coli</i> (Le1680)
XXI: <i>E. coli</i> (Le0840)	XXII: <i>E. coli</i> (Le1035)	XXIII: <i>E. coli</i> (Le0040)	XXVI: <i>E. coli</i> (Le4968)	XXV: <i>E. coli</i> (Le4805)
XXVI: <i>E. coli</i> (Le4306)	XXVII: <i>E. coli</i> (Le4074)	XXVIII: <i>E. coli</i> (Le3917)	XXIX: <i>E. coli</i> (Le4588)	XXX: <i>E. coli</i> (Le4888)
XXXI: <i>E. coli</i> (Le4798)	XXXII: <i>E. coli</i> (Le4983)	XXXIII: <i>E. coli</i> (Le0022)	XXXIV: <i>E. coli</i> (Le0311)	



29 **Figure S1. The *Lysobacter* genes was screened to identify targets that block AHL**
 30 **production in *E. coli* JM109 expressing the active AHL synthase gene *pcoI*. The**
 31 34 selected *Lysobacter* genes were those only conservatively shared by
 32 non-AHL-producing *Lysobacter* but absent in AHL-producing species. Its details are
 33 shown in the Table S1 with red highlight. PcoI is an AHL synthase from
 34 *Pseudomonas fluorescence* 2P24.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

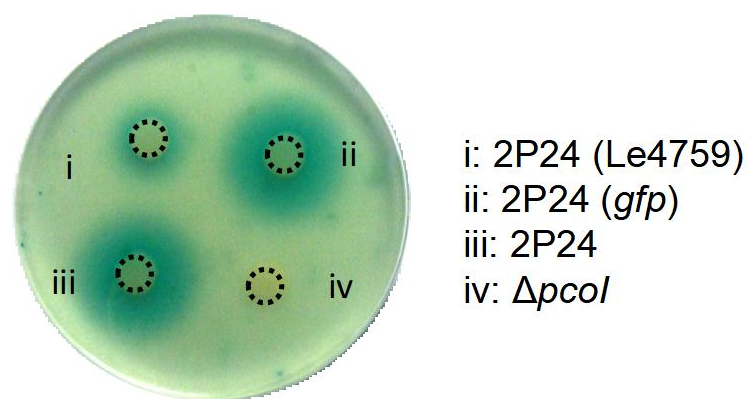


Figure S2. Representative images showing the production of AHL in *Pseudomonas fluorescence* 2P24 expressing Le4759. AHL production was determined by the AHL biosensor JZA1, and the AHL quantification data were shown in Figure 1F.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

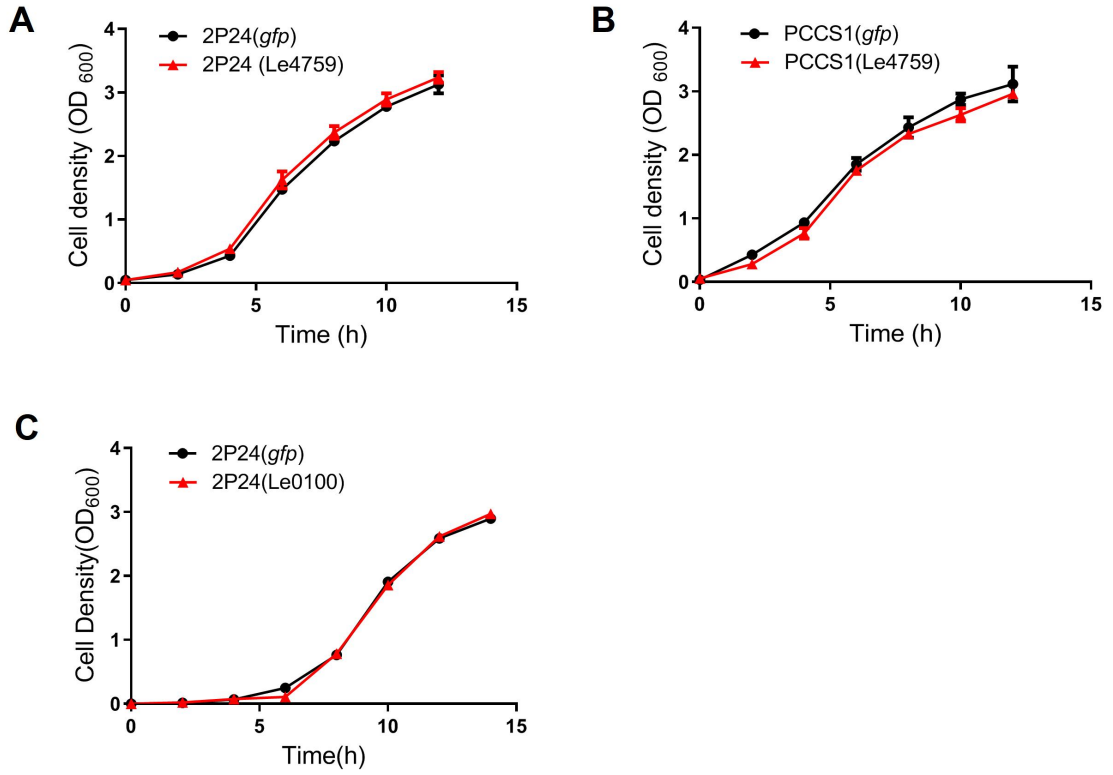


Figure S3. Heterogeneous expression of *Le4759* and *Le0100* in *Pseudomonas fluorescence* 2P24 did not alter the normal growth ability of the bacteria.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23

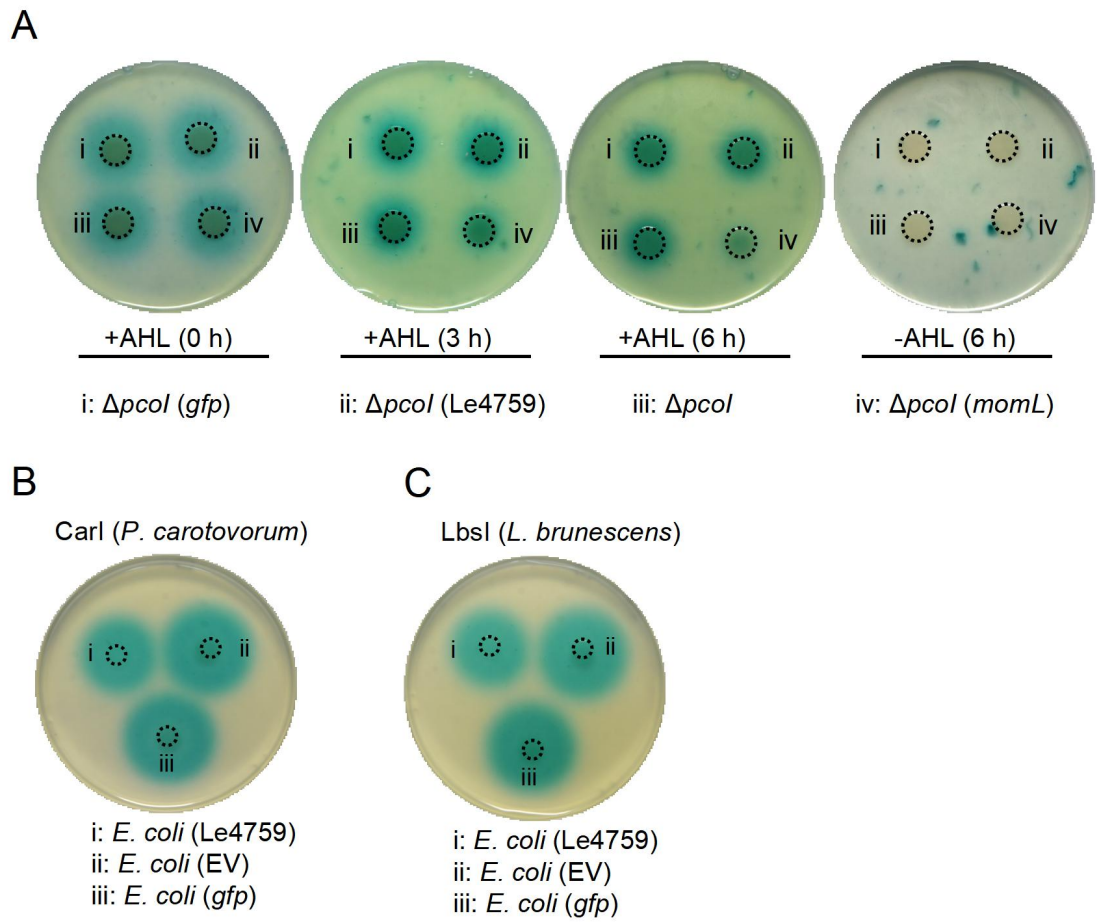
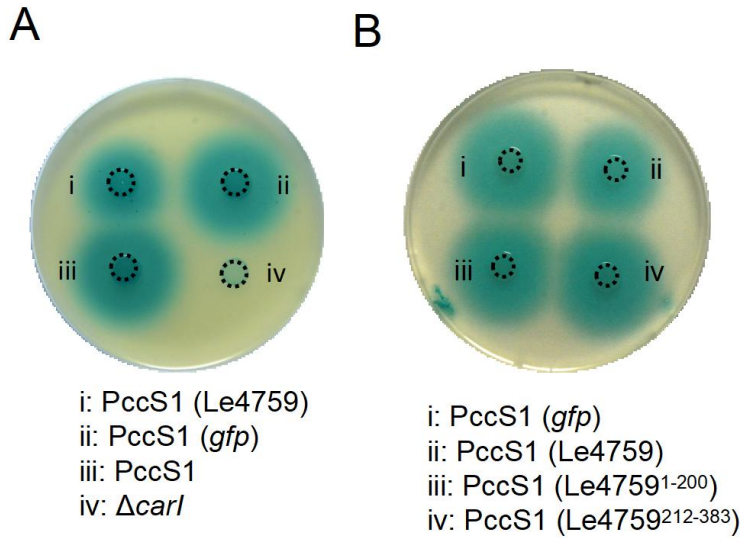
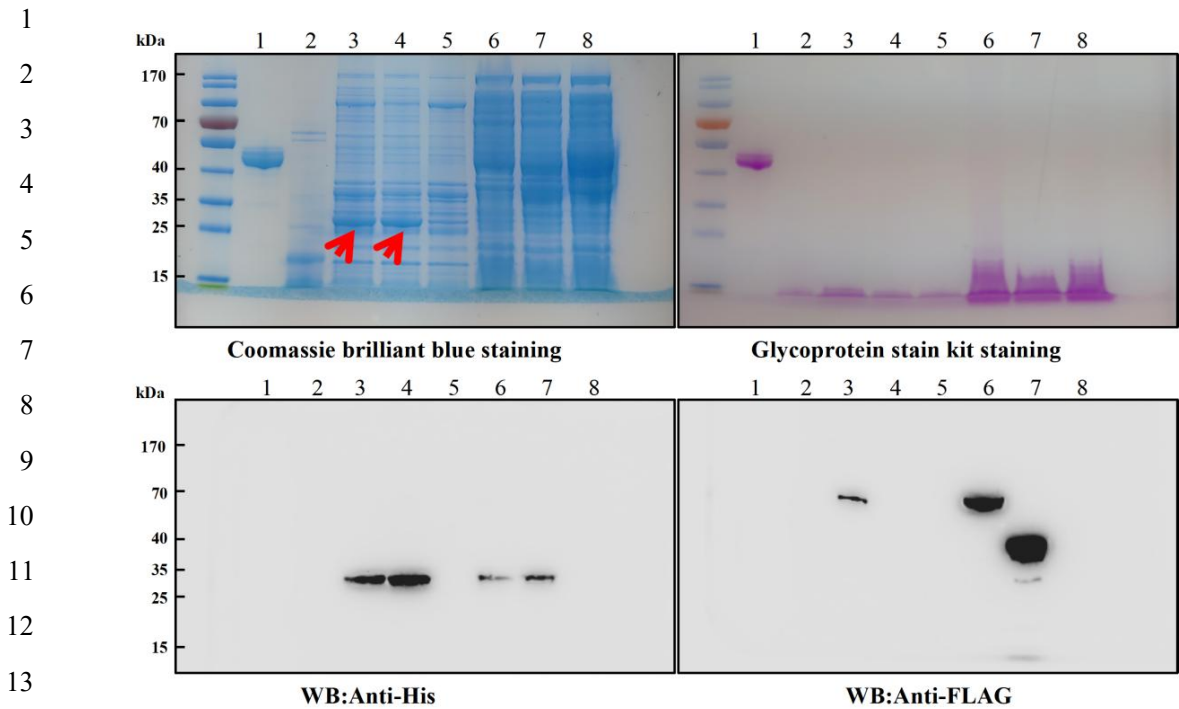


Figure S4. Representative images of AHL production as determined by the AHL biosensor JZA1. (A) Le4759 failed to degrade the commercial AHL standard (3-oxo-C10-HSL). **(B-C)** Le4759 blocked the CarI- or LbsI-dependent AHL production in *E. coli*. AHL quantification data in panels A, B and C were shown in Figure 2A, 2F and 2G, respectively.

1
2
3
4
5
6
7
8
9
10
11

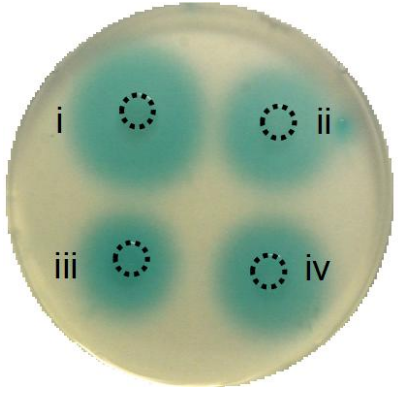


12 **Figure S5. Representative images showing AHL production in *Pectobacterium***
13 ***carotovorum* PccS1 expressing Le4759 and its two truncated variants. AHL**
14 **production was determined by the AHL biosensor JZA1. AHL quantification data in**
15 **panels A and B were shown in Figure 3A and 3G, respectively.**



15 **Figure S6. Co-expression of the *Le4759* and *pcoI* gene in *E. coli* did not detect the**
 16 **glycosyltransferase activity of *Le4759*.** We co-transformed the pUCP-PcoI-His and
 17 pBBR-*Le4759*-FLAG or pBBR-GFP-FLAG (negative control) in *E. coli* DH5 α , and
 18 drove the expression of *pcoI* and *Le4759* by a constitutive promoter from the
 19 corresponding plasmid. The corresponding strains were collected and broken by
 20 ultrasound, and the supernatant was incubated with anti-His magnetic beads to
 21 successfully enriched the PcoI-His protein under the condition of expressing *Le4759*
 22 and GFP. Glycoprotein staining assay revealed that *Le4759* did not appear to be an
 23 active glycosyltransferase for glycosylated PcoI in *E. coli*. Line1: Positive control,
 24 containing 16% glycoprotein; Line2: Negative control, no glycoprotein; Line3-5 :
 25 PcoI+*Le4759*, PcoI+GFP, *E. coli* DH5 α supernatant enriched with anti-His magnetic
 26 beads, respectively; Line6-8 : PcoI+*Le4759*, PcoI+GFP, *E. coli* DH5 α cell
 27 fragmentation supernatant, respectively. The red arrow indicates the position of the
 28 PcoI.

1
2
3
4
5
6
7
8
9
10
11
12
13



i: PccS1 (*gfp*)
ii: PccS1 (Le4759)
iii: PccS1 (La1165)
iv: PccS1 (GLE2246)

Figure S7. Representative images showing the production of AHL in *Pectobacterium carotovorum* PccS1 expressing Le4759 and its two homologous genes (La1165 and GLE2246). AHL production was determined by the AHL biosensor JZA1, and AHL quantification data were shown in Figure 3H.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

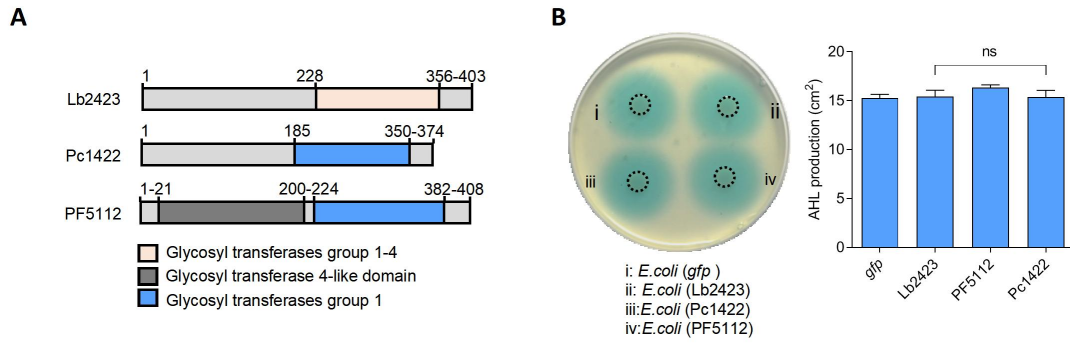
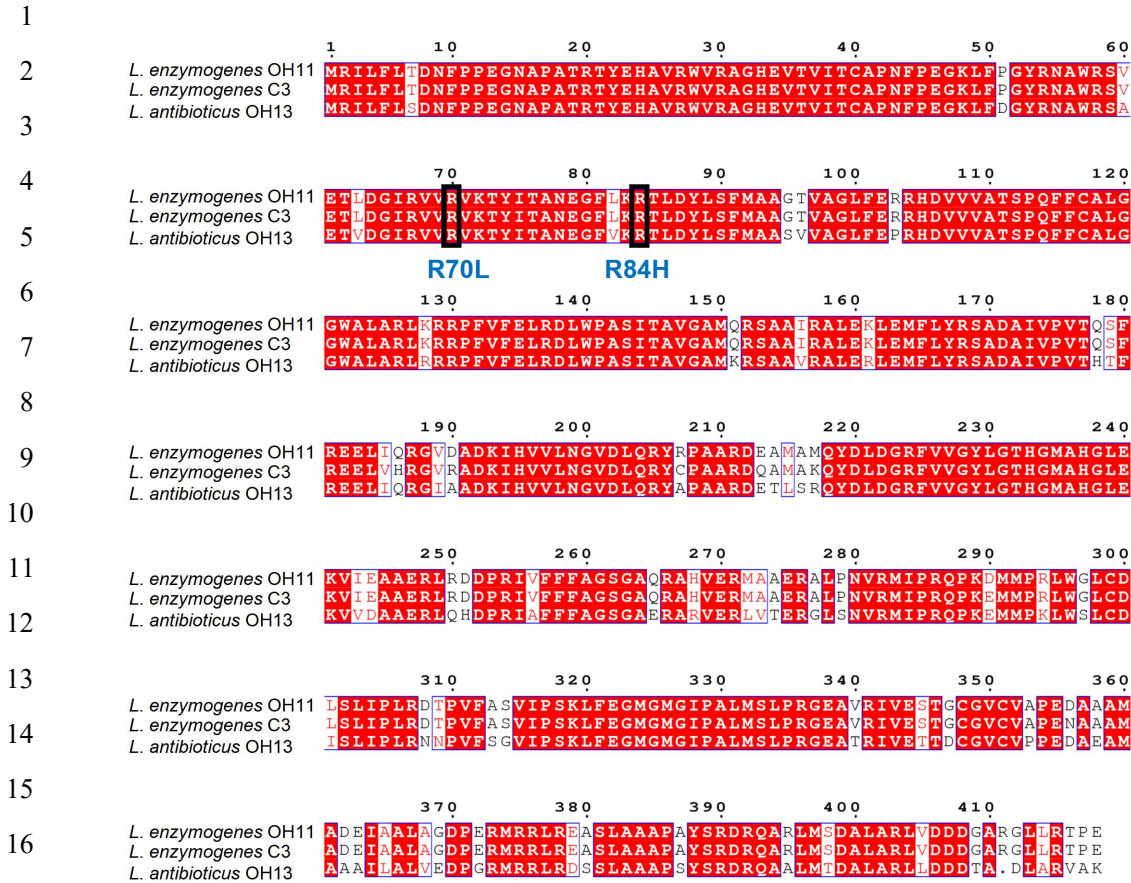


Figure S8. Detection of AHL-quenching activity of three Le4759-unrelated, glycosyltransferase-domain containing genes. (A) Prediction domains of three Le4759-unrelated, glycosyltransferase-domain containing genes (*Lb2423*, *Pc1422* and *PF5112*) of *Lysobacter brunescens* OH21, *Pectobacterium carotovorum* PccS1 and *Pseudomonas fluorescens* 2P24. (B) Ectopic expression of the *Lb2423*, *Pc1422* and *PF5112* gene in recombinant, *pcoI*-expressing *E. coli* had no detectable AHL-quenching activity. Recombinant *E. coli* was induced to produce AHL by adding 0.1% arabinose. NCBI reference sequence accession numbers: WP_194431416.1 (*Pc1422*), WP_134925924.1 (*PF5112*).



18 **Figure S9. Sequence alignment of Le4759 and its two homologues (GLE2246 and**

19 **La1165).** Boxes indicates the positions of conserved amino residues R70 and R84.

20 GLE2246 and La1165 are homologues of Le4759 from the non-AHL-producing *L.*

21 *enzymogenes* C3 and *L. antibioticus* OH13, respectively.

1
2
3
4
5
6
7
8
9
10
11
12
13

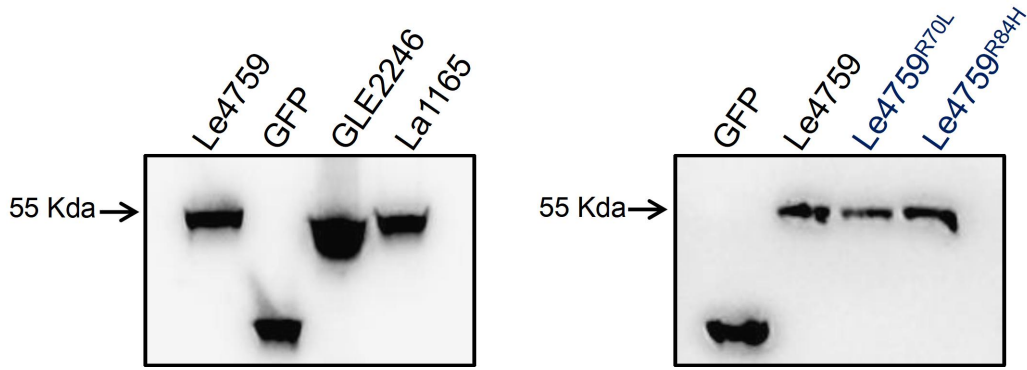
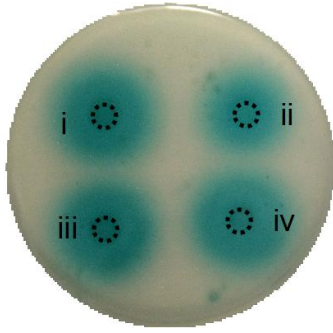


Figure S10. Western blotting analysis of protein expression in *Pectobacterium carotovorum* PccS1. The left panel represents the heterogeneous expression of Le4759 and its two homologous genes (*GLE2246* and *La1165*) in *P. carotovorum* PccS1. The right panel represents the expression of two Le4759 point mutant variants (Le4759^{R70L} and Le4759^{R84H}) in *P. carotovorum* PccS1.

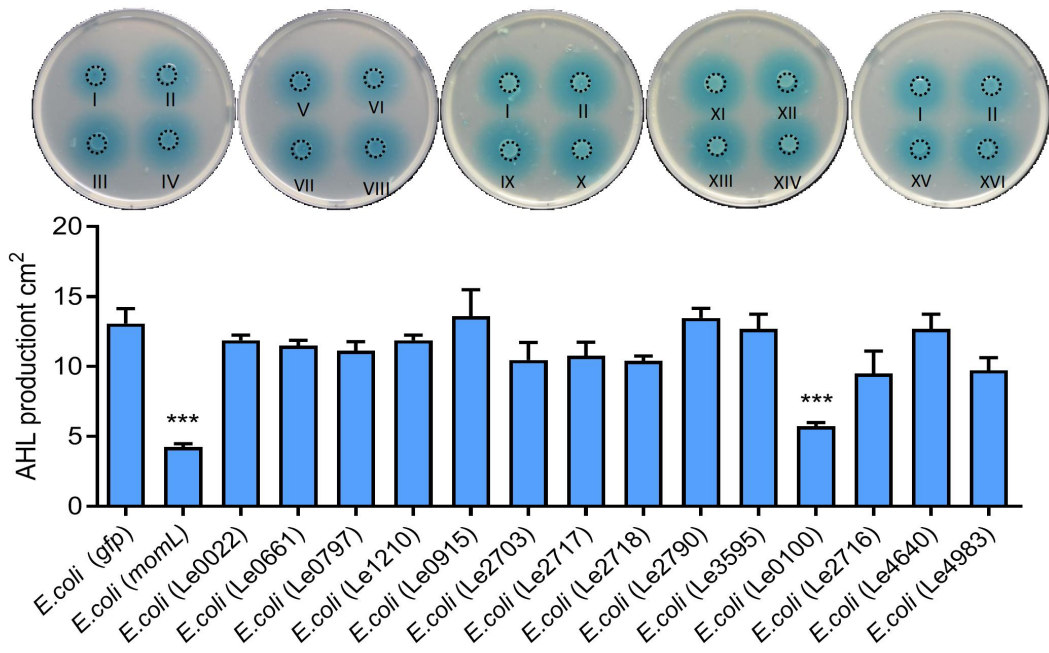
1
2
3
4
5
6
7



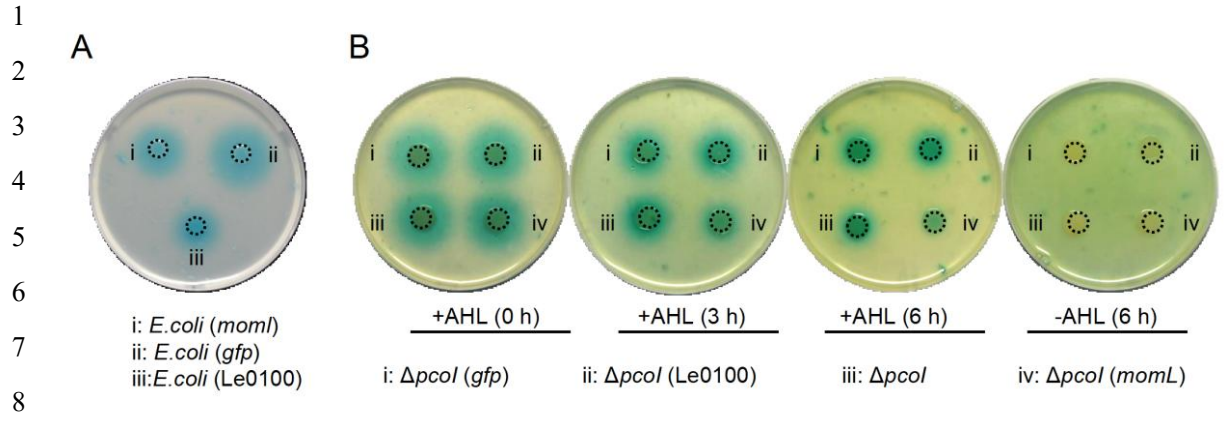
i: PccS1 (*gfp*)
ii: PccS1 (Le4759)
iii: PccS1 (Le4759^{R70L})
iv: PccS1 (Le4759^{R84H})

8 **Figure S11. Representative images showing the production of AHL in**
9 ***Pectobacterium carotovorum* PccS1 expressing Le4759 and its two point mutant**
10 **variants.** AHL production was determined by the AHL biosensor JZA1, and the AHL
11 quantification data were shown in Figure 3I.

1 I: *E. coli* (*momL*) II: *E. coli* (*gfp*) III: *E. coli* (Le0022) IV: *E. coli* (Le0661) V: *E. coli* (Le0797) VI: *E. coli* (Le1210)
 2 VII: *E. coli* (Le0915) VIII: *E. coli* (Le2703) IX: *E. coli* (Le2716) X: *E. coli* (Le2717) XI: *E. coli* (Le2718)
 3 XII: *E. coli* (Le2790) XIII: *E. coli* (Le3595) XIV: *E. coli* (Le4640) XV: *E. coli* (Le0100) XVI: *E. coli* (Le4983)

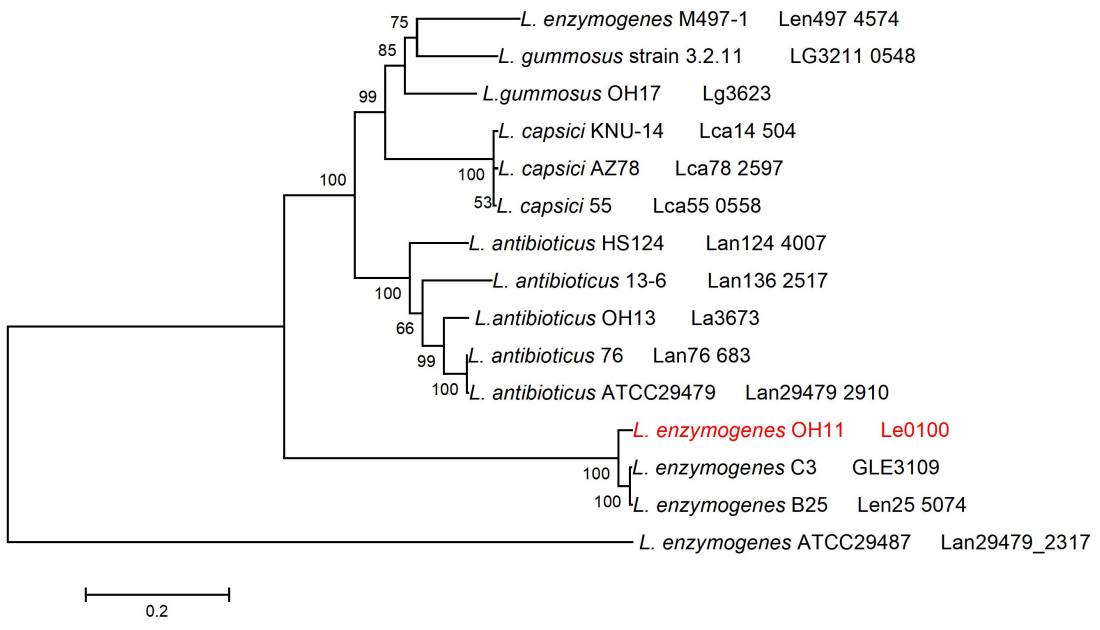


16 **Figure S12. Additional 14 *Lysobacter* genes were screened to identify targets that**
 17 **could prevent AHL production in *E. coli* JM109 expressing the active AHL**
 18 **synthase gene *PcoI*.** The 14 *Lysobacter* genes selected were those that were
 19 conservatively shared only by non-AHL-producing *Lysobacter* but not present in
 20 AHL-producing species. Its details were shown in a supplemental excel table with
 21 blue highlight. *PcoI* is an AHL synthase from *Pseudomonas fluorescence* 2P24.



9 **Figure S13. Representative images of AHL production as determined by the AHL**
10 **biosensor JZA1. (A)** Le0100 blocked the PcoI-dependent AHL production in *E. coli*.
11 **(B)** Le0100 failed to degrade the commercial AHL standard (3-oxo-C10-HSL). AHL
12 quantification data in panels A and B were shown in Figure 4A and 4B, respectively.

1
2
3
4
5
6
7
8
9
10
11
12
13



14 **Figure S14. Phylogenetic tree of Le0100 and its homologues from 15 selected**
15 **non-AHL-producing *Lysobacter* species.**

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

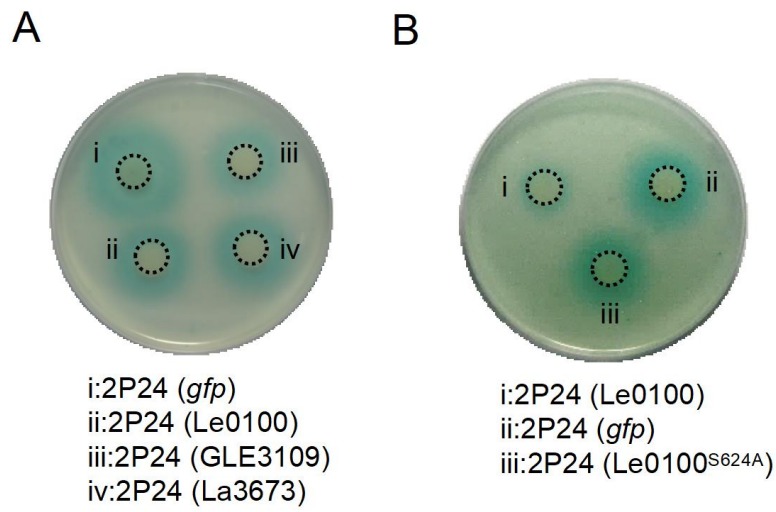
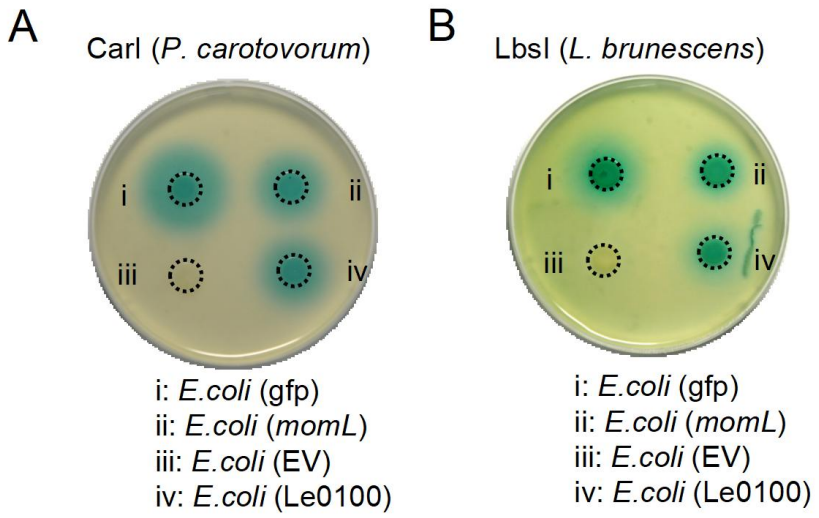


Figure S15. Representative images showing AHL production of *Pseudomonas fluorescence* 2P24 expressing Le0100 and its two homologous genes (A) as well as its point mutant variant (B). AHL quantification data in panels A and B were shown in Figure 4C and 4D, respectively.

1
2
3
4
5
6
7
8
9
10
11



12 **Figure S17. Representative images showing production of AHL in *E. coli***
13 **co-expressing Le0100 with the AHL synthase CarI (A) or LbsI (B) gene. AHL**
14 **production was determined by the AHL biosensor JZA1. AHL quantification data in**
15 **panels A and B were shown in Figure 4E and 4F, respectively.**

1 **References**

- 2 1. Qian, G., Hu, B., Jiang, Y. & Liu, F. Identification and characterization of
3 *Lysobacter enzymogenes* as a biological control agent against some fungal
4 pathogens. *Agricultural sciences in China* 8:68-75.
5 [https://doi.org/10.1016/S1671-2927\(09\)60010-9](https://doi.org/10.1016/S1671-2927(09)60010-9).
- 6 2. Wei, H., and L. Zhang. 2006. Quorum-sensing system influences root colonization
7 and biological control ability in *Pseudomonas fluorescens* 2P24. *Antonie van*
8 *Leeuwenhoek* 89:267-280. <https://doi.org/10.1007/s10482-005-9028-8>.
- 9 3. Zhu, J., Y. Chai, Z. Zhong, S. Li, and S. C. Winans. 2003. Agrobacterium bioassay
10 strain for ultrasensitive detection of N-acyl homoserine lactone-type
11 quorum-sensing Molecules: Detection of Autoinducers in *Mesorhizobium huakuii*.
12 *Applied and Environmental Microbiology* 69:6949-6953.
13 <https://doi.org/10.1128/AEM.69.11.6949-6953.2003>.
- 14 4. Xu, G., S. Han, C. Huo, K. Chin, S. Chou, M. Gomelsky, G. Qian, and F. Liu. 2018.
15 Signaling specificity in the c-di-GMP-dependent network regulating antibiotic
16 synthesis in *Lysobacter*. *Nucleic Acids Research*.
17 <https://doi.org/10.1093/nar/gky803>.
- 18 5. Kovach, M. E., P. H. Elzer, D. Steven Hill, G. T. Robertson, M. A. Farris, R. M.
19 Roop, and K. M. Peterson. 1995. Four new derivatives of the broad-host-range
20 cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. *Gene*
21 166:175-176. [https://doi.org/10.1016/0378-1119\(95\)00584-1](https://doi.org/10.1016/0378-1119(95)00584-1).
- 22 6. Liao, J., D. Shen, L. Lin, H. Chen, Y. Jin, S. Chou, X. Yu, T. Li, and G. Qian. 2021.
23 Bacterial quorum sensing quenching activity of *Lysobacter* leucyl aminopeptidase
24 acts by interacting with autoinducer synthase. *Computational and Structural*
25 *Biotechnology Journal* 19:6179-6190. <https://doi.org/10.1016/j.csbj.2021.11.017>.

26

27